THE ECOLOGY OF FUNGI INFECTING UNTREATED AND PRESERVATIVE-TREATED SAPWOOD OF *Pinus radiata* D. DON

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Summary

The succession of fungi in untreated stakes differed in above-ground, ground-line and below-ground zones. These differences could be related to changes in wood moisture content and source of infection: slight changes of pH were of no significance. Above-ground the succession was from primary moulds to bluestain fungi. At ground-line and below it was from primary moulds to soft-rot fungi to secondary moulds and primary Basidiomycetes (white-rot fungi). The pattern of succession at the ground-line was completed after 3–4 months' exposure, but below-ground it was much slower. In contrast, the succession of fungi in stakes treated with copper-chrome-arsenate was similar in all zones and fewer fungi were involved. The frequency of occurrence of individual fungi was related to their tolerance of the preservative and its components. At ground-line and below the succession of infections was similar to that in untreated wood, but soft-rot was not observed until the third month and decay was not widespread even after 18 months' exposure. Growth studies of fungi were of little value in explaining the basis for the succession.

Species lists and mean frequencies of fungi in all zones of both sets of stakes are provided together with the results of decay capability tests.

INTRODUCTION

This paper provides basic information on the succession of fungi colonizing untreated and treated (copper-chrome-arsenate preservative) sapwood of *Pinus radiata* D. Don in above-ground, ground-line and below-ground zones.

The general principles governing the colonization of plant debris and wood have been discussed by Garrett (1955, 1963) and Findlay (1966). Detailed studies of this decomposition process, however, have largely been concerned with substrates other than wood. Also, most of the work on wood has been restricted to studies of the colonization by fungi of unbarked timber in above-ground situations. There have been investigations of fungal colonization on logs, logging-slash and natural debris (Spaulding and Hansbrough, 1944; Chesters, 1950; Mangenot, 1952; Toole, 1965; Ueyama, 1966), fire-killed trees (Basham, 1957, 1958), windthrown trees (Stillwell, 1959; Englehart, 1961) and pulpwood bolts (Findlay, 1940; Atwell, 1956; Shigo, 1962; Henningsson, 1967). Shigo (1967) reviews work on the successions of organisms in discolouration and decay of wood with particular reference to the living tree.

Successions of fungi on debarked timber or timber in contact with the soil have received far less attention. Corbett and Levy (1963) outlined the following general pattern of colonization on untreated pine posts: Moniliales gp I (*Penicillium* spp., *Trichoderma viride, Botrytis* sp.): Sphaeropsidales (soft-rot fungi); Moniliales gp II (*Gliocladiopsis* sp., *Cylindrocarpon* sp., *Mennomiella* sp.); Basidiomycetes (*Coprinus* sp. and unidentified spp.). A similar pattern of colonization based on types of infection (mould to soft-rot to decay) was described by Merrill and French (1966). The diversity of fungal species encountered in the early stages of colonization was illustrated by Käärik (1967) who described the flora of untreated pine and spruce posts after exposure for 6 months. In particular, at least 26 Basidiomycetes were described of which some species (*Poria rixosa* (Karst.) Karst., *Fomes annosus* (Fries) Cooke, *Odontia bicolor* (Alb. and Schw. ex Fr.) Fr., *Pleurotus mitis* (Pers. ex Fr.) Sing. and *Polyporus amorphous*, Fr.) were responsible for above-ground infections.

In contrast, successions of fungi on preservative-treated timber have received no attention. Some work has been done in describing the micro-fungi associated with treated wood (Greaves and Savory, 1965), but most has been concerned with delimiting the preservative tolerances of those fungi directly responsible for decay. These tolerances have been described for many basidiomycete species (Zabel, 1954; Baechler and Roth, 1956; Cowling, 1957; Young, 1961; DaCosta and Kerruish, 1964) and also for strains of individual species (Gersonde, 1958; Schultz, 1958). In general, soft-rot fungi have been shown to be more tolerant of preservatives than Basidiomycetes (Savory, 1955; Scholles, 1957; Duncan, 1960; DaCosta and Kerruish, 1963). Data on the tolerances of mould and stain fungi are less readily obtained, but work concerned with the degradation of wood preservatives yields some information on the response of these fungi to various toxicants. Thus, certain mould fungi have been shown to detoxify arsenical compounds (Gozio, 1892; Challenger, 1945), phenyl mercuric acetate (Russel, 1955), ammoniacal copper arsenate (Duncan and Deverall, 1964), fluor-chrome-arsenate-diphenol (Madhosingh, 1961a, Duncan and Deverall, 1964), pentachlorophenol (Duncan and Deverall, 1964; Cserjesci, 1967; Unligil, 1968) and creosote (Christensen *et al.*, 1942; Marsden, 1954). The role such microfungi may play in the decay process of preservative-treated wood has been demonstrated by Madhosingh (1961b).

The present investigation was undertaken to study the development of the fungal flora on untreated and preservative-treated wood: the first to describe the normal process of decay and the second to see how the presence of a preservative may modify this process. In many ways treated wood could be regarded as a 'selective-bait' for preservative-tolerant fungi.

The necessity for this work may be summarized by a statement of Shigo (1967) who said, 'organisms must be understood both as individuals and as part of an ecosystem'. A wealth of information has been published about the individual fungi which cause decay and discolourations of timber (Cartwright and Findlay, 1958; Findlay, 1959; Duncan, 1960; Duncan and Lombard, 1965), but the ecology of these organisms has received little attention.

MATERIALS AND METHODS

The experimental material consisted of 68 stakes (14 in × 1¼ in × 1¼ in) of untreated *Pinus radiata* sapwood and an equivalent number of stakes treated to refusal with a copper-chrome-arsenate preservative (approximate formulation as commercial salt: $CuSO_4$, 22·4%; $Na_2Cr_2O_7$, 39·0%; $As_2O_5 . 2H_2O$, 33·4%; $Na_4As_2O_7$, 5·2%) by the Bethal process to give retentions ranging from 0·095–0·15 lb/cu ft. Stakes were air-dried to a moisture content of 15% and placed at random in an experimental plot (soil a grey-brown sandy loam of pH 5·2 to 5·6 developed on pumice). Two stakes were removed when they were sampled weekly and 3 stakes when sampled less frequently: intervals between sampling dates were increased periodically with time. Untreated stakes were studied for 1 year and treated stakes for 18 months.

When removed from the plot stakes were surface-cleaned with 95% ethanol and then cut into the various cross-sectional zones as shown in Fig. 1. Isolations were made from each zone of the stake, from both just beneath the surface and internally, using the 'two-chisel' technique (Greaves and Savory, 1965). A minimum number of 8 isolations was made from each zone, half of which were plated onto Abrams (1948) medium plus 10 g/l cellulose powder and half onto 2% malt agar. Other 'selective' media were used initially, but to no advantage. Results of isolations were expressed in terms of frequency of occurrence. Frequency was taken as the number of positive isolations of a fungus expressed as a percentage of the total number of isolations made from each zone of all stakes at a particular sampling date. Changes in the moisture content and pH of the wood were also studied.

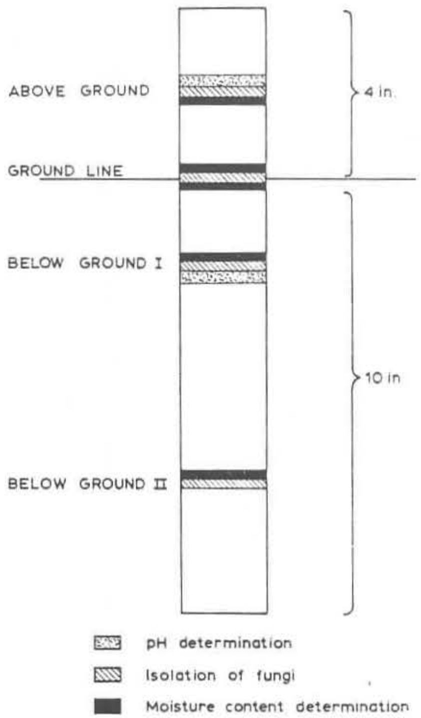

Fig. 1. Position of the various samples taken from stakes.

Decay capability tests were made with all of the Basidiomycetes obtained in pure culture using the soil-wood block exposure technique (American Society for Testing and Materials, 1961). Similarly, 25 fungi were tested to determine their capability of producing soft-rot by exposing veneers of *Fagus sylvatica* L. on Abrams medium in flat, screw-cap jars previously inoculated with the fungus. In both methods decay was estimated as a mean percentage loss of weight, but soft-rot was also confirmed by microscopic examination.

The influence of temperature and pH on the growth rate of all fungi obtained in pure culture was also studied. For temperature studies fungi were placed on 2% agar and then incubated at 10°, 15°, 20°, 25°, 30° and 35°C. The same medium modified with a citric acid–potassium phosphate buffer system (Strider and Winstead, 1960) provided nominal pH values from 3–10 inclusively; cultures were incubated at 25°C. Growth was measured as daily increment (mm) in colony radius. Most fungi isolated from treated stakes, and some fungi isolated only from untreated stakes, were tested in agar-plate culture to assess their tolerance to the preservative and its components. The solution of the toxicant(s) and the agar were sterilized separately, mixed together when still warm and then poured. Concentrations were also adjusted so that the agars differed only in the amount of toxicant(s) present.

RESULTS

Succession of infections

Bluestain appeared on the above-ground portions of untreated stakes within the first 2 weeks of exposure. It penetrated fairly rapidly into the wood, but showed no further development after the eighth week. At ground-line, the presence of soft-rot in the sixth week was the first indication of fungal attack. This infection was superficial (less than $\frac{1}{16}$ inch–$\frac{1}{8}$ inch penetration) and its presence was obscured soon after the onset of normal basidiomycetic decay in the third month. Decay originated as small, localized patches of bleached wood and penetrated the stakes in the direction of the wood-rays where it was observed as light brown-discoloured streaks or wedges. After 1 year the entire cross-section of stakes at the ground-line was affected by this decay. Below-ground the succession of infections was similar, but much slower. Soft-rot was first detected in the third month and was not obscured by the onset of basidiomycete infection until the ninth month.

In preservative-treated stakes bluestain was also the first sign of fungal attack above-ground, but this was not observed until the fifth month of exposure. Although there was an increase in the incidence of infection, bluestain did not penetrate deeply into the stakes. At ground-line and below soft-rot was detected after three months. It increased in severity until the ninth month when all regions of the stake in contact with the soil were affected. Soft-rot remained superficial (approximately $\frac{1}{8}$ inch penetration). Below-ground, small regions of decay were detected on certain stakes after 1 year's exposure. Although decay became more frequent in the ensuing six months it was never observed on all 3 stakes of a sample. In general, this type of infection remained superficial. Decay was not encountered in ground-line zones.

Table I

MEAN FREQUENCIES OF FUNGI IN ABOVE-GROUND, GROUND-LINE AND BELOW-GROUND ZONES OF UNTREATED STAKES

FREQUENCY OF FUNGI*

	Above-ground (Months of Exposure)				*Ground-line (Months of Exposure)*				*Below-ground (Months of Exposure)*			
	0–3	4–6	7–9	10–13	0–3	4–6	7–9	10–13	0–3	4–6	7–9	10–13
ACTINOMYCETES												
Streptomyces sp	0	0	0	0	1	2	0	0	0	0	0	0
Unidentified spp.	0	0	0	0	0	1	0	0	0	0	0	0
PHYCOMYCETES												
Absidia sp.	0	0	0	0	1	1	2	1	2	2	2	2
Mucor spinescens	0	1	0	0	0	1	1	0	1	1	2	1
Mucor sp.	0	2	1	0	0	0	0	1	0	1	1	1
Zygorhyncus moelleri	0	0	0	0	1	1	0	0	4	3	4	3
Unidentified sp.	0	0	0	0	1	4	2	1	1	2	1	2
ASCOMYCETES												
Ceratocystis sp.	0	0	0	0	0	1	0	0	0	0	0	0
Chaetomium globosum	0	1	0	0	0	1	1	0	0	0	0	0
Yeasts	1	1	0	0	1	0	0	0	0	0	0	0
Unidentified sp.	0	0	0	0	0	0	0	0	1	0	0	0
BASIDIOMYCETES												
Trametes cinnabarina	0	0	0	0	1	0	0	0	1	0	0	0
Trechispora brinkmanni	0	0	0	0	0	1	0	0	0	0	0	0
Unidentified sp. No. 16	0	0	0	1	0	2	2	0	0	1	0	0
Unidentified sp. No. 17	0	1	0	0	0	0	0	0	0	0	0	0
Unidentified sp. No. 18	0	0	0	0	0	1	0	0	0	0	0	0
FUNGI IMPERFECTI												
Moniliales : Moniliaceae												
Botrytis sp.	0	0	0	0	0	0	0	0	1	0	0	0
Cephalosporium sp.	1	0	1	0	0	1	1	0	1	1	1	1
Dactylaria sp.	0	0	0	0	0	1	0	0	0	0	0	0
Gliocladium catenulatum	0	1	0	0	1	2	0	0	1	2	3	1
Gliocladium sp.	0	0	0	0	1	0	0	0	1	0	0	0
Moeszia sp.	0	0	0	0	1	0	1	0	1	1	1	0
Oidium sp.	0	0	0	0	0	1	0	0	0	0	0	0
Paecilomyces sp.	0	0	0	0	1	1	0	0	1	1	1	0
Penicillium spp.	1	3	3	3	1	3	1	3	1	2	2	2
Trichoderma viride	3	5	5	5	4	5	6	5	4	5	5	5
Verticillium sp.	0	1	0	0	1	2	1	0	1	2	3	1
Dematiaceae												
Alternaria tenuis	2	1	1	1	1	1	1	0	0	1	1	0
Aureobasidium pullulans	1	1	0	1	0	0	0	0	0	0	0	0
Cladosporium herbarum	3	1	0	0	1	1	0	1	0	1	0	0
Helminthosporium sp.	1	1	0	0	1	0	0	0	1	0	0	0
Hormiscium sp	0	1	1	1	0	1	0	0	0	1	0	0
Tuberculariaceae												
Cylindrocarpon sp.	0	0	0	0	1	1	0	0	0	0	0	0
Epicoccum nigrum	4	3	3	1	2	1	0	0	1	1	0	0
Fusarium spp.	2	1	2	1	4	4	4	1	4	5	4	4
Sphaeropsidales												
Aposphaeria sp.	1	0	0	0	0	0	0	0	0	0	0	0
Coniothyrium sp.	0	0	0	0	1	0	0	0	0	1	0	0
Diplodia pinea	1	0	0	0	0	0	0	0	0	0	0	0
STERILE MYCELIA												
White colonies	1	2	2	2	2	3	3	3	2	2	2	1
Dark colonies	1	1	3	1	1	1	0	0	0	1	1	0

* Mean frequency of occurrence 0 = 0%; 1 = 0–5%; 2 = 5–10%; 3 = 10–25%; 4 = 25–50%; 5 = 50–75%; 6 = 75–100%.

Succession of fungi

(*a*) *Untreated stakes*

Major fluctuations in the frequencies of individual fungi are summarized in Table I. The figures shown for each fungus are based on the mean values of percentage frequency within each 3-month period. In all 3 zones the number of species increased over the first 6 months of exposure. This was followed by a more rapid decline during the succeeding 3 months, after which the number of species remained fairly constant. The greatest number of species was recorded from the ground-line zone and the fewest from above-ground.

In above-ground zones *Epicoccum nigrum* Link was both the primary colonizer and the dominant fungus over the intial 3 months of exposure. Bluestain fungi were first encountered in the third week, reached a peak of activity in the sixth week and then gradually their numbers declined. By the end of the fourth month they were encountered sporadically. *Cladosporium herbarum* Link ex Fr. was the main bluestain fungus with *Alternaria tenuis* Nees sub-dominant. Minor bluestain fungi consisted of the remaining members of the Dematiaceae. As these fungi decreased in numbers there was a corresponding increase amongst the Moniliaceae which became the dominant group by the fifth month. *Trichoderma viride* Pers ex. Fr., first isolated after the main peak for bluestain had passed, did not become prominent until *Epicoccum nigrum* had begun to decline. *Penicillium* spp. were relatively late colonizers and often of local importance. Other groups of fungi were of no numerical importance.

At ground-line, *Fusarium* spp. and *Epicoccum nigrum* were the main primary colonizers, but the latter was encountered rarely after this initial period of activity. From the third week *T. viride* was dominant to the *Fusarium* spp. Secondary mould infection (*Gliocladium* spp., *Penicillium* spp., *Verticillium* sp. and some members of the Mucorales) occurred mainly between the third and ninth months and actinomycetes between the third and sixth months. The main Basidiomycete isolated (designated Basidiomycete No. 16: possibly *Polyporus semipileatus*. Peck) was first obtained from decaying regions of stakes in the fourteenth week. It was encountered regularly until the ninth month after which it could not be isolated although decay was well established.

In below-ground zones *Zygorhyncus moelleri* Vuillemin (Mucorales) was the dominant fungus during the first month of exposure, but was then replaced by *T. viride* to become a sub-dominant with *Fusarium* spp. Secondary mould infections, similar to those at ground-line, occurred from the third month onwards.

(*b*) *Treated stakes*

In general, member of the Moniliaceae were the main elements of the flora in all 3 zones of the stakes (Table II). They were most active below-ground and least active above-ground. *Cephalosporium* sp. and *Penicillium* spp. (principally *P. frequentens*, Westling) were amongst the first colonizers of the wood and also the dominant fungi throughout the entire experimental period. *Trichoderma viride* was a common member of the flora below-ground, but was encountered only sporadically in above-ground and ground-line zones until the last 6 months of exposure. Dematiaceous fungi were of little importance apart from *Cladosporium elatum* (Harz) Nannfeldt which was isolated from all zones. It was obtained in relatively large numbers during the first year of exposure, but declined in the succeeding 6 months. Other fungi, apart

Table II

MEAN FREQUENCIES OF FUNGI IN ABOVE-GROUND, GROUND-LINE AND BELOW-GROUND ZONES OF TREATED (CuCrAs PRESERVATIVE) STAKES

	FREQUENCY* OF FUNGI																	
	Above-ground (Months of Exposure)						*Ground-line (Months of Exposure)*						*Below-ground (Months of Exposure)*					
	0–3	4–6	7–9	10–12	13–15	16–18	0–3	4–6	7–9	10–12	13–15	16–18	0–3	4–6	7–9	10–12	13–15	16–18
ACTINOMYCETES																		
Streptomyces sp.	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0
MUCORALES																		
Mucor spinescens	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Mucor spp.	0	0	1	0	0	0	1	1	0	0	0	1	1	0	0	0	0	0
ASCOMYCETES																		
Chaetomium globosum	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Yeasts	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
BASIDIOMYCETES																		
Unidentified spp.	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0
FUNGI IMPERFECTI																		
Moniliales : Moniliaceae																		
Acremonium sp.	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Aspergillus fumigatus	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Candida albicans	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
Cephalosporium sp.	3	4	5	4	5	4	2	3	4	3	3	4	3	4	4	5	6	6
Gliocladium sp.	1	1	0	0	1	0	0	0	0	0	1	1	1	1	0	1	1	0
Moeszia sp.	0	0	0	0	0	0	1	0	0	0	0	0	1	2	1	2	1	1
Penicillium spp.	2	4	5	3	4	4	3	5	4	4	4	4	5	6	6	4	5	5
Trichoderma viride	1	1	0	0	3	0	1	1	0	0	3	1	2	4	3	4	5	3
Verticillium sp.	1	0	0	0	1	0	1	1	1	0	1	1	1	2	0	2	0	3

* Mean frequency of occurrence 0 = 0%; 1 = 0–5%; 2 = 5–10%; 3 = 10–25%; 4 = 25–50%; 5 = 50–75%; 6 = 75–100%.

(*cont.*)

Table II—*continued*

	0–3	4–6	7–9	10–12	13–15	16–18	0–3	4–6	7–9	10–12	13–15	16–18	0–3	4–6	7–9	10–12	13–15	16–18
Dematiaceae																		
Alternaria tenuis	0	0	0	0	0	0	1	0	1	0	0	1	1	0	0	0	0	0
Aureobasidium pullulans	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0
Cladosporium elatum	3	3	2	1	2	0	2	1	2	1	0	0	3	2	1	3	0	1
Cladosporium herbarum	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Curvularia lunata	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Phialophora sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0
Stemphylium ilicis	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Tuberculariaceae																		
Cylindrocarpon sp.	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Epicoccum nigrum	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fusarium spp.	0	0	0	0	0	0	1	1	1	1	0	1	1	1	0	3	2	0
Sphaeropsidales																		
Unidentified spp.	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0
STERILE MYCELIUM																		
White colonies	2	2	1	1	1	1	1	1	0	2	0	1	2	3	0	3	3	3
Dark colonies	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0

from *Fusarium* spp. and *Verticillium* spp. which were frequent in below-ground zones, were of minor importance. Over the last 6 months basidiomycetes were isolated once from ground-line and twice from below-ground. These fungi were not obtained in pure culture.

Fluctuations of moisture content and pH

In both untreated and treated wood moisture content was highest below-ground and lowest above-ground. In general, values were highest in untreated wood; above-ground moisture content fluctuated between 30–70 % (O.D.W.) and below-ground fluctuated between 50–90 % reaching 110 % by the end of 1 year compared with a range of 25–40 % and 50–85 % respectively in untreated wood. Moisture content at ground-line closely followed that of below-ground zones in untreated wood and that of above-ground zones in treated wood.

Preservative-treated stakes were slightly less acid (pH 5·6) than untreated stakes (pH 5·4) at the beginning of the experiment. Apart from minor fluctuations, pH values in treated stakes were little changed after exposure for 18 months. In contrast, untreated stakes became more acid with time, the decrease in pH being most marked in below-ground zones. During the 12 months of exposure mean values fell from pH 5·4 to 4·9.

Decay capability tests

Under the conditions of the soil–wood block test none of the Basidiomycetes isolated from untreated stakes caused any severe loss of weight in the *Pinus radiata* sapwood blocks after incubation for 10 weeks. *Trametes cinnabarina* (Jacq. ex Fr.) Fr. (= *Coriolus sanguineus*) and the unidentified Basidiomycete 16 were responsible for a 12·2 % and a 10·1 % loss of weight respectively. The remaining unidentified Basidiomycetes (17 and 18) caused weight losses of between 5–10 %, but *Trechispora brinkmanni* (Bres) Rogers and Jackson had no measurable effect on the blocks.

Table III

SOFT-ROT CAPABILITY TESTS

Fungus	*Wt. loss* (%)	*Fungus*	*Wt. loss* (%)
Streptomyces sp.	47·8	*Aureobasidium pullulans*	0·0
Chaetomium globosum	57·7	*Cladosporium elatum*	2·5
Aspergillus fumigatus	0·0	*Cladosporium herbarum*	0·0
Botrytis sp.	0·0	*Helminthosporium* sp.	2·4
Cephalosporium sp.	29·2	*Hormiscium* sp.	4·8
Gliocladium catenulatum	0·0	*Stemphylium ilicis*	3·1
Moeszia sp.	3·5	*Fusarium* sp.	0·0
Paecilomyces sp.	0·0	*Aposphaeria* sp.	0·0
Penicillium frequentens	0·0	*Coniothyrium* sp.	43·9
Penicillium sp.	0·0	*Diplodia pinea*	0·8
Trichoderma viride	0·0	Culture A.10	0·0
Verticillium	0·0	Culture A.25	0·0
Alternaria tenuis	1·7		

* Loss of weight based on the mean values of two or four veneers of *Fagus sylvatica* incubated with the fungus for eight weeks at 25°C.

The oxidation reaction of the mycelium of these Basidiomycetes with various phenolic compounds showed that all belonged to the group IIIB fungi of Käärik (1965)—the true white-rot fungi—apart from *T. brinkmanni* which was a brown-rot fungus.

Soft-rot capability tests (Table III) showed that at least 4 species could produce this type of decay. These fungi, *Streptomyces* sp., *Chaetomium globosum*, Kunze, *Cephalosporium* sp. and *Coniothyrium* sp., all caused large losses of weight in the test pieces of beech veneers. Soft-rot was confirmed by microscopic examination. There was slight evidence of erosion channels in the secondary cell walls of veneers infected with *Moeszia* sp. With the exception of *Coniothyrium* sp., which was recorded only from untreated wood, the above fungi were isolated from both sets of stakes. *Helicosporium aureum* (Corda) Linder, a known soft-rot fungus, was recorded as sporulating on an untreated stake in the eighteenth week. It was not possible to isolate this fungus.

Physical factors and the growth of fungi

The optimum temperature for the growth of most fungi was 25°C. *Chaetomium globosum*, one isolate of *Cephalosporium* sp., *Curvularia lunata* (Walker) Boedijn and *Paecilomyces* sp. had optima between 25°C–30°C and *Cladosporium elatum*, two further isolates of *Cephalosporium* sp. and *Aspergillus fumigatus* had optima above 30°C. *Alternaria tenuis, Botrytis* sp., *Cladosporium herbarum*, *Hormiscium* sp. and *Moeszia* sp. produced optimum growth between 20°C–25°C.

Similarly most fungi produced optimum growth at the same pH (5·0–6·0). Exceptions were *Aureobasidium pullulans*, *Cladosporium herbarum* sp. and one isolate of *Cephalosporium* sp. which produced maximum growth between pH 4·0–5·0 together with *Absidia* sp., *Chaetomium globosum* and *Helminthosporium* sp. with optima between pH 6·0–7·0. Tolerance to a comparatively wide range of pH (at least 4·0–9·0) was general amongst most fungi, but a few had more specific requirements. In particular, the main Basidiomycete isolated (No 16) produced measurable growth only between pH 4·0–6·5

Comparative studies of rates of growth showed that the Mucorales (with the exception of *Absidia* sp), *Botrytis* sp and *Trichoderma viride* were the fastest growing fungi (daily increment in colony radius of at least 10 mm). The slowest growing fungi (less than 5 mm per day) comprised *Absidia* sp., the remaining members of the Moniliaceae, *Fusarium* sp., *Cladosporium elatum*, *C. herbarum*, *Coniothyrium* sp. and Basidiomycete 16. The last fungus together with *Cephalosporium* sp. and *Moeszia* sp. had a daily increment of less than 2 mm. The remaining fungi formed an intermediate group producing from 5–10 mm per day of mycelial growth.

Agar-plate toxicity tests

The results of testing the tolerance of certain fungi to the preservative and its individual components are shown in Table IV. The fungi most commonly isolated from treated wood had the greatest tolerance to the preservative and those isolated only from untreated wood (mainly Basidiomycetes) had the least tolerance. Tests with the individual components of the preservative showed that the major elements of the flora of treated wood were most tolerant of arsenic and copper and least tolerant of chromium. The concentrations of copper sulphate required to inhibit growth of *Cephalosporium* sp.,

Table IV

TOLERANCE OF CERTAIN FUNGI TO COPPER, CHROMIUM AND ARSENIC

Fungus	*Relative Importance*		*Concentrations bracketing total inhibition point for mycelial growth*				
	Treated Wood	*Untreated Wood*	*Cu ppm (as $CuSO_4$)*	*Cr ppm (as $Na_2Cr_2O_7$)*	*As ppm (as As_2O_5)*	*As ppm (as $Na_4As_2O_7$)**	*CuCrAs ppm (as preservative)*
Cephalosporium sp.	Very common	Frequent	2,000–3,200	0–40	0–325	20,000+	500–1,000
Penicillium frequentens	Very common	Common	4,000–4,400	200–400	22,000–32,000	20,000+	1,500–2,000
Cladosporium elatum	Common	Absent	6,000–6,600	40–100	10,000–13,000	20,000+	2,500–3,000
Penicillium sp.	Common	Common	1,200–1,600	400–600	3,250–6,500	20,000+	3,000+
Fusarium sp.	Frequent	Very common	400–600	100–200	1,300–1,600	20,000+	500–1,000
Moeszia sp.	Frequent	Occasional	400–600	100–200	1,000–1,300	20,000+	500–1,000
Trichoderma viride	Frequent	Very common	200–400	40–100	650–1,000	12,000–20,000	500–1,000
Verticillium sp.	Frequent	Frequent	40–100	200–400	1,000–1,300	20,000+	250–500
Cladosporium herbarum	Occasional	Common	600–800	40–100	325–650	2,000–2,500	500–1,250
Aspergillus fumigatus	Rare	Absent	400–600	0–40	1,000–1,300	20,000+	250–500
Alternaria tenuis	Rare	Frequent	200–400	0–40	1,000–1,300	20,000+	500–1,250
Mucor spinescens	Rare	Frequent	200–400	40–100	650–1,000	20,000+	500–1,250
Stemphylium ilias	Rare	Absent	200–400	0–40	325–650	20,000+	250–500
Chaetomium globosum	Rare	Rare	100–200	0–40	160–325	20,000+	250–500
Epicoccum nigrum	Rare	Common	40–100	100–200	0–65	40–100	0–250
Coniothyrium sp.	Absent	Rare	100–200	0–40	0–65	200–400	0–250
Paecilomyces sp.	Absent	Occasional	40–100	0–40	1,000–1,300	20,000+	0–250
Zygorhyncus moelleri	Absent	Common	0–40	0–40	1,000–1,300	400–600	0–250
Trametes cinnabarina	Absent	Rare	0–40	0–40	0–65	40–400	0–250
Trechispora brinkmanni	Absent	Rare	0–40	0–40	160–325	100–200	0–250
Unidentified basidiomycete 16	Absent	Frequent	0–40	0–40	65–160	100–200	0–250
Unidentified basidiomycete 17	Absent	Rare	40–100	0–40	0–65	0–40	0–250
Unidentified basidiomycete 18	Absent	Rare	0–40	0–40	160–325	100–250	0–250

* No explanation could be given for the high As tolerances recorded with this chemical. Consequently, most attention should be focussed on tolerances to As_2O_5, the main arsenic component of the preservative.

Penicillium frequentens and *Cladosporium elatum* (the main fungi isolated from treated wood) were particularly high when compared with other fungi. In general, tolerance to the chemicals decreased as the frequency of fungi in treated wood decreased. With minor exceptions, fungi isolated rarely from treated wood or only from untreated wood were the most sensitive to the chemicals under test.

DISCUSSION

The succession of fungi described is based mainly on the results of periodic isolations. The influence that both isolation technique and culture media have on the number and type of fungi isolated is well known (Warcup, 1960; Greaves and Savory, 1965). For this reason undue emphasis should not be placed on the relative frequencies of occurrence of individual fungi which may be more a measure of ease of isolation. In addition, it is not known whether the fungus isolated was in a physiologically active or inactive state within the wood and therefore a particular organism may be assigned more importance than is warranted.

The succession of infections in the various zones of untreated wood (blue-stain to moulds, above-ground and mould to soft-rot to decay at ground-line and below) confirms the results of previous workers (Shigo, 1962; Corbett and Levy, 1963; Merrill and French, 1966).

Colonisation of untreated wood above-ground did not proceed beyond the mould phase in the present study. Earlier work suggests that Basidiomycetes would have followed this mould infection in time (Shigo, 1962; Toole, 1965; Henningsson, 1965, 1967; Butcher, 1967). Corbett and Levy (1963) recorded soft-rot fungi from the rain-soaked tops of fence posts, but soft-rot is not likely to be a major cause of decay unless moisture content of the wood remains very high as in wood-chip piles (Lindgren and Eslyn, 1961; Butcher and Howard, 1968).

At ground-line the succession of organisms was more complete, but stain fungi were of no significance possibly because of a lack of inoculum. During the initial development of the moulds (*Trichoderma viride*, *Fusarium* spp.) soft-rot fungi colonized the stakes, but their activity was soon curtailed as a result of subsequent invasions by other fungi—secondary moulds (*Gliocladium* spp., *Penicillium* spp., *Verticillium* sp.) and Basidiomycetes. The Basidiomycetes invaded the wood when fungal activity, and consequently competition for available food and space, was at its greatest. Below-ground, the succession of fungi was much slower and the flora more restricted. It was, apart from the increased importance of Mucorales, essentially similar to that at ground-line.

Difficulties were experienced in isolating soft-rot fungi because infection was superficial and the affected zone was invaded by all other fungi. Although four species (*Cephalosporium* sp., *Chaetomium globosum*, *Coniothyrium* sp. and *Streptomyces* sp.) were shown to produce this decay the possibility of other fungi being involved should not be overlooked. Similar problems of isolation occurred with the Basidiomycetes, particularly over the last 3 months of exposure when the numbers of Moniliaceae were high, but the additional problem of identification of Basidiomycetes was also experienced. Little is known about the soil-borne Basidiomycetes associated with the decay on wood and until recently *Trechispora brinkmanni* (Mangenot and Reymond, 1963; Merrill and French, 1966) and a *Coprinus* sp. (Corbett and Levy, 1963) were the only species identified. For this reason the work of Käärik (1967) is of particular value as it lists 21 identified species of Basidiomycetes as colonizing wood after exposure for 6 months, of which 12 were isolated from ground-line or below-ground zones. In the present study only 5 Basidiomycetes were isolated from stakes (*Trametes cinnabarina, Trechispora brinkmanni*, unidentified Basidiomycetes 16 (possibly *Polyporus semipileatus*), 17 and 18. It appears that the primary Basidiomycete colonizers of wood in contact with the soil are not usually those fungi first to be isolated from timber in above-ground situations: *e.g. Schizophyllum commune*, Fr., *Stereum purpureum* (Pers) Fr., *S. hirsutum* (Wills) Pers., *S. complicatum* (Fr.) Fr., *Trametes cinnabarina* (Chesters, 1950; Atwell, 1956; Toole, 1965; Findlay, 1966; Ueyama, 1966). A similar observation was made by Käärik (1967).

In general, softwoods fail mechanically as a result of attack by a brown-rot fungus. In the experimental area the main decay fungi isolated from failed untreated stakes and posts of *Pinus radiata* are *Poria vaillantii* (Fr.) Cooke and *Trametes serialis* Fr. However, with the exception of *Trechispora brinkmanni* all of the Basidiomycetes isolated from test stakes were white-rot fungi and the majority of decay fungi isolated from pine and spruce poles by Käärik (1967) were of this type. It appears that white-rot fungi may precede brown-rot fungi in the colonization of untreated softwood timber in ground-contact.

With preservative-treated stakes the general pattern of succession was similar to that on untreated wood, although much slower. At ground-line and below moulds were succeeded by soft-rot fungi in the third month. Decay was observed sporadically after 1 year in below-ground zones, but not elsewhere. The fungal flora of treated wood was very restricted and not greatly influenced by position along the stake. Thus, the flora from above-ground zones was essentially the same as that from below-ground. *Penicillium* sp. and *Cephalosporium* sp. were co-dominant in all zones and were the main primary colonizers with *Cladosporium elatum*. No other fungi were of importance apart from *Trichoderma viride*, *Fusarium* spp. and *Verticillium* sp. in below-ground zones after exposure for 1 year. The soft-rot stage in the succession was of comparatively long duration (over 1 year) and analysis of population figures and results of soft-rot capability tests suggest that *Cephalosporium* sp. was the main agent of this degrade. In contrast, Basidiomycetes played a very minor role in the colonization of treated wood over the 18-month period of study.

In untreated stakes it was concluded that difference in wood moisture content was one of the main factors influencing the pattern of colonization in the various zones. Above-ground, moisture content was too low for invasion by soft-rot or Basidiomycetous fungi and below-ground it was too high for the succession to progress beyond the soft-rot stage (decay recorded from this region was usually the result of a downward extension of decay from the ground-line zone). At ground-line conditions were favourable for growth of most fungi: in addition, this zone was liable to attack from both airborne and soilborne fungi. The slight differences of pH recorded in stakes is unlikely to have influenced the succession to any degree as most fungi were tolerant of a fairly wide range of pH. Comparison of rates of growth for individual fungi shed little light on the basis of the successional pattern. In fact, it appeared that Basidiomycetes successfully colonized the wood in spite of their growth characteristics rather than because of them. It is unlikely that their success can be solely attributed to an adaptation to a changing (nutrient) substrate, particularly as a further invasion by moulds occurred at the same time. The moulds were not capable of utilizing cell wall components.

With treated wood the limiting factor for colonization by fungi was undoubtedly the presence of the preservative. This was demonstrated by the results of agar-toxicity tests where tolerance to the preservative and its components was, in general, related to the frequency of occurrence of individual fungi. Differences in the moisture content of the wood had no apparent effect, although decay was limited to the below-ground, namely wetter zones.

This work indicates the value of ecological studies in obtaining a true appreciation of the decay process in wood (treated or untreated). It is essential that decay should be regarded as the end result of the activity of successive populations of fungi rather than as a result of invasion by an individual fungus.

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